CURRENT STATE OF SMALLPOX PROPHYLAXIS

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[Following is the translation of an article by S. S. Marennikova and V. D. Solovyev, Moscow Institute of Viral Preparations and the Central Institute for the Advancement of Doctors, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), Vol 42, No 9, 1965 pages 15--21. It was submitted on 7 July 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

For a number of reasons, up until the present time smallpox remains in the ranks of important problems of contemporary public health. The importance of this problem is determined by 4 factors: The latitude of distribution of the disease and the large number of recorded cases (see the table), the severity of the course of the disease and the possibility of the infection entering into countries where it has already been liquidated.

Of particular importance for countries where it has already been liquidated is the possibility of the infection being brought in from endemic regions. In recent years there has not been one year free from smallpox being brought into the countries of Europe. Moreover, the number of smallpox cases in Europe as a result of the infection being brought in has even increased. In the last five years smallpox has been noted in Belgium, the Federal Republic of Germany, the German Democratic Republic, Hungary, Poland, Spain, Sweden, Switzerland, England and the Soviet Union (1960). The particular danger of smallpox being brought into our country is conditioned not only by our unusually extensive cultural and economic ties with practically all the countries of the world, but also by the lengthiness of land borders, a significant part of which belong to countries with unfavorable conditons in respect to smallpox.

In connection with this it is completely apparent that in research and practical works on smallpox we should cover a vast circle of problems; along with work on the improvement of smallpox vaccine and vaccine prophylaxis, it is necessary to study the smallpox causative agent, methods for its rapid indication, means for the emergency prophylaxis and therapy of smallpox infection, etc. Particular attention should be turned to the study of the peculiarities of the epidemiology of imported outbreaks of smallpox and the development of correct antiepidemic measures which are adequate for the new conditions.

The study of the smallpox causative agent, which up until recently belonged to the ranks of poorly investigated viruses, has moved forward significantly in recent years. Data has been obtained on its ultrastructure, pathogenicity for laboratory animals, behavior in tissue culture, antigenic and other properties (Bykovskiy et al., 1961; Marennikova, 1961, 1963; Marennikova et al., 1963; Bykovskiy, 1964). In the course of these investigations an experimental model of smallpox infection has been developed on young white mice (Kaptsova, 1963, 1964). It should be stressed that the need

of an experimental model of smallpox infection is especially great at the present time. It is necessary not only for the study of the pathogenesis of smallpox infection and antismallpox immunity, but also for evaluating the means of chemoprophylaxis of smallpox.

The study of the properties of the smallpox virus made it possible to arrive at the development of criteria for the differentiation of separate representatives of the group of smallpox viruses and the creation of new, more modern, methods for laboratory diagnosis.

As regards the first of these problems, it is necessary to note that all the existing classifications of smallpox viruses differ from each other, both in the number of species included in this group as well as in the criteria for separating different representatives into independent species (alastrim virus, vaccine virus, cowpox, etc.). To a significant degree this is conditioned by general difficulties which are encountered in the taxonomy of viruses, and in particular with the difficulties of defining the idea of species. However, in a number of cases the stated deviations are the result of an inadequate familiarity with one or the other causative agent.

In recent years the laboratory diagnosis of smallpox has advanced considerably, and certain original methods which were developed in the USSR were included in the recommendations for laboratory diagnosis which were proposed in the beginning of 1964 by the Committee of Experts on Smallpox of the World Health Organization. However, it would be incorrect to consider this problem resolved. Thus, the results, recently obtained at the Moscow Institute of Viral Preparations, of a comparative study of the sensitivity of various methods of indication of the smallpox virus when it is applied to the chorioallantoic membrane of a chick embryo, showed that the introduction of the material according to the method of Westwood guaranteed the detection of the virus in those concentrations in which it was not determined if the infection was carried out on the chorioallantoic membrane from the side of the air sac. New methods for the indication of the smallpox antigen in a tissue culture on the basis of using the immunofluorescence method were demonstrated by Avakyan et al. (1961) and investigated in detail by Gurvich and Roykhel (1964). This method, just as the method of exposing Guarnieri's bodies in tissue culture (Marennikova et al. 1963), makes it possible to detect the presence of the virus in the course of the first 6--12 hours.

Among the problems which are connected with the investigation of the smallpox virus, the problem concerning the mutability of this virus and the possibility of its transformation into a vaccine virus is very interesting. The problem concerning the possibility of such a transformation served as the object of more than a century's searches, carried out by the scientists of many countries in which smallpox vaccine was produced. However, in spite of a tremendous number of works this problem was still not resolved conclusively. The majority of investigators admitted the possibility of the transformation of the smallpox virus into a vaccine virus. It should be noted, however, that all these works were conducted prior to the time that the smallpox virus was obtained in a pure culture, and therefore, the stated results cannot be viewed as completely reliable. In connection with this the necessity arose for checking these investigations on a contemporary level. However, all our attempts at transforming the smallpox virus into a vaccine, which were carried out on various biological objects

(chick embryos, rabbits, white mice and tissue culture) and with the use of various strains of the virus, ended in failure in every case. Recently Herrlich (1958) and Herrlich et al. (1963) reported analogous results.

The stated data should be critically attributed to the hypothesis, according to which the majority of the vaccine strains which are used in production originate from the virus of natural smallpox. As is known, together with this a supposition is expressed concerning the origination of the vaccine virus from the virus of genuine cowpox. Even in view of the fact that the strains obtained by Jenner are lost at the present time there are reports by a number of investigators who soon after Jenner obtained the materials for inoculations from animals or people who had cowpox. A comparative study of the viruses of genuine cowpox and the vaccine showed, however, that there were noticeable differences between them, including in antigen structure and morphological changes which are caused by them in susceptible tissues in the nature of intracellular inclusions. In their time Downie and Haddock (1952) and van Tongeren (1952) simultaneously isolated variants of the cowpox virus which based on certain features -- the absence of a hemorrhagic component -- were closer to the vaccine virus than the former earlier known strains of the cowpox virus. A detailed study of such a variant, conducted recently at the Moscow Institute of Viral Preparations, made it possible to detect a number of new interesting facts. In particular it turned out that if the cowpox virus can be clearly differentiated from the vaccine virus based on the absence of a main precipitation band in the double diffusion reaction in gel with antivaccine serum, then a "white" variant of this virus is in no way different from strains of the vaccine virus. Similar data were obtained during the cross investigation in the reactions of neutralization and hemagglutination inhibition. A cytological investigation of tissue cultures which were infected with the "white" variant of the cowpox virus showed that in the cells there was almost a complete absence (0.8%) of eosinophilic inclusions which are specific for cowpox. These findings point to the possibility of the existence of natural variants of cowpox which are similar to the vaccine virus.

The production of small pox vaccine is an affair which is sufficiently old and developed, and everything relative to investigations in this area would seem to be only of theoretical interest. Together with this the experience of recent years testifies that even here there are still many unresolved problems which have primary practical importance. We will dwell on several examples. It was discovered in 1959 that the smallpox vaccine which is prepared at the Gamaleya Institute of Microbiology and Epidemiology is heterogeneous in a genetic respect. It has at least two different variants of the vaccine virus -- a "white" and a "surface", or "gray, diffuse." It was subsequently ascertained that all the vaccines produced in the USSR were genetically heterogeneous, that is, in other words they cannot be acknowledged as standard in the full sense of the word. These findings served as impetus for carrying out a vast front of works, started at the Mosow Institute of Viral Preparations, directed at the isolation and study of pure clones from genetically heterogeneous vaccines and the obtaining of a genetically homogeneous vaccine (Marennikova and Maltseva, 1963; Maltseva

and Marennikova, 1963; Chernos and Gendon, 1963). At the present time, genetically homogeneous vaccines from the "white" clone have been obtained and approved in a number of plants of the USSR (Moscow Institute of Viral Preparations, Tashkent and Tomsk Institutes of Vaccines and Sera, Belorusskiy Institute of Epidemiology and Microbiology). The detailed characteristics of clones, isolated both from native as well as from several foreign, genetically heterogeneous, vaccines have also been established (Maltseva and Marennikova, 1963). These investigations showed that the properties of a vaccine are determined by the properties of the clone which is predominant in it, regardless of whether it is "white" or "surface." In connection with this the properties of the clone which is found in less proportion are as if concealed and a further study of such clones is of specific interest. The obtaining of genetically homogeneous vaccines makes it possible to eliminate the reasons for the non-standardization of a preparation. Together with this it is necessary to clearly realize that this does not appreciably change the quality of the strains -- the spectrum of their pathogenicity for animals and reactogenicity.

The production of smallpox vaccine can be summed up mainly in that if not in all then in the majority of cases each vaccine producing laboratory used "its own" strain. As a result of this at the present time there is a large collection of vaccine strains which have been selected by means of accidental findings or directed mutability. These strains differ from each other in origin, method of passaging, reactogenicity, etc. There might be strains which are of significant interest among them, however, no serious attempts have been made at a comparative evaluation of the various industrial strains. Such a work has begun just recently. It turned out that among the strains investigated there are indeed strains which are of importance for production.. The most interesting in this respect is strain E-3/59 which has been studied in detail (Marennikova and Maltseva, 1963; Genkina et al., 1964). This strain is genetically homogeneous, weakly pathogenic for laboratory animals, thermostable, and highly immunogenic. At the present time there is already information characterizing its behavior under experimental-industrial conditions and following the vaccination of persons. The resulting data make it possible to evaluate this strain positively and recommend it for subsequent approval and use. Along with this, the investigations carried out do not exhaust the possibility of improving our industrial strains. Further work is necessary of the selection and directed mutability of strains by means of influencing the genetic base of the virus in order to obtain a vaccine strain which possesses a high immunogenic potentiality and a minimum reactogenicity.

The greater the demands we set forth for the vaccine strain and the preparation which is prepared from it, the more accurate, reliable and stable should be the methods for controlling the vaccine. However, the main methods for control of activity (methods of Grot and Gins), which have been used extensively in our country up until recently, do not answer modern requirements. The methods of titrating on the chorioallantoic membrane of a chick embryo and in tissue culture, which were proposed already several years ago (Marennikova and Stepanova, 1958; Marennikova and Osenina, 1959; Archakov et al., 1962), only now are beginning to win a place which is appropriate for their significance. For the standardization of the smallpox vaccine which is produced in the USSR it is necessary to create a national standard for this preparation

To a significant degree the results of vaccination depend on the method of administration of the preparation. As is known, only one method of vaccination is used in the USSR -- the linear scarification of the skin with 3 incisions. Nevertheless, in a number of countries other methods are used: The method of scarification of the skin with one incision, the method of multiple pressure, or as we call it the method of multiple pricking, circular scarification with the help of a rotary lancet, intracutaneous administration of the vaccine, etc. For the USSR, where yearly up to 50 million vaccinations and revaccinations are performed, the problem of the utilization of the most adequate method of vaccination is very urgent. The selection of a method for administering the vaccine has attracted attention recently in other countries also, especially there where measures are being carried out for the liquidation of smallpox by means of mass vaccination.

Of the methods which are utilized at the present time, most interest lies in scarification with linear incisions and multiple pricking. An analysis of available data makes it possible to consider that the immunity which is created by inoculation depends on the number of incisions made. According to observations which were carried out over the last 10--12 years in India, among smallpox victims the smallest percentage is made up of persons having 4 inoculation scars. In this group lethality from smallpox is significantly lower (0.6%) than with persons with a lesser number of incisions; the greatest lethality (11.7%)was in persons with one scar. Together with this, several negative aspects of using a large number of incisions are known. Thus, with a large number of incisions there is an increase in the severity of the course of the vaccination reaction. This fact is especially sharply exposed during the vaccination of impoverished and weakened children, for example, in the former colonial or dependent countries.

In analyzing the problem of the number of incisions, it should be mentioned that the successful campaign for liquidating smallpox in several countries of South America was realized by carrying out inoculations with one incision. The Committee of Experts on Smallpox from the World Health Organization considered it feasible to recommend the use of only one incision for the initial vaccination.

The multiple pressure or multiple prick method, proposed by Leake already in 1927, has acquired all the more advocates in recent years. From the point of view of practical application it has a number of qualities: Less possibility of trauma, greater standardization of procedure, etc. Along with this, data on a comparative evaluation of the effectiveness of this method and the scarification method are scarce and do not always conform. In the Soviet Union up until recently the multiple prick method has been ignored. Only 2 years ago investigations were begun on the approbation of this method and a comparison with the scarification method (Abidov, 1964; Ivanov and Konstantinova, 1964). Encouraging results have been obtained in the course of these investigations.

Over the last years there has been a considerable increase in

the collection of vaccines which are used for the prophylaxis of viral and bacterial infections. The necessity for the alternate administration of various vaccines has extremely complicated and prolonged the process of inoculative prophylaxis, and made it cumbersome and expensive. Therefore, work on the study of the feasibility of associated vaccination against smallpox and other infections is very timely. In particular, favorable results have been obtained in the case of the simultaneous application of smallpox vaccine and the BCG vaccine, vaccines against smallpox, tularemia and plague, a mixed vaccine against smallpox and yellow fever, etc. These investigations, which in our country are still in the initial stage of development, must be speeded up and expanded in every possible way. Of course here it is necessary to take into consideration the epidemiological expediency of combining antigens and to comprehensively study the inoculative immunity which is created by associated vaccines.

While devoting a great deal of attention to problems of virological investigations and smallpox vaccine, we should simultaneously expand the experimental study of the immunology of smallpox, since there is much which is unclear in the determination of the immunoreactivity of an organism, the evaluation of the role of tissue and humoral factors, and the understanding of cellular reactions which emerge following the introduction of the virus. The condition of allergy, which is connected with antismallpox vaccinations, has hardly been studied at all.

In summing up the results of a few works on the mechanism of antismallpox immunity, which have been carried out in the Soviet Union for sometime, it can be considered substantiated that the synthesis of antismallpox antibodies is realized in organs which are rich in macrophage--lymphoid tissue - the spleen and lymph nodes. The production of antibodies depends on the dose of the virus and the method of applying it, which determine the degree of involvement of the lymphoid organs in the process of antibody formation. With a sufficient amount of virus in the blood, which is achieved experimentally by intravenous immunization, this process takes place most intensively due to the maximum envelopment of immunologically competent cells and tissues. Following the intracutaneous administration of a small dose of the virus, the formation of antibodies takes place only in the regional lymph node. It must also be assumed that following the vaccination of man the intensity and the duration of the acquired immunity is connected with the intensity of the dissemination of the virus in the inoculated organism, with the intensity of its engagement by the cells of the lymphoid tissue or, in other words, with the individual immunoreactivity. The investigations by Gulevich (1962) showed that not only antibodies have an importance in antismallpox immunity; the cells of lymphoid tissue, extracted from the organism of a preliminarily immunized animal or obtained following contact of the virus in vitro with a transplanted line of cells of the reticular type, acquired a resistance which was transmitted to subsequent generations of the same cells. In the end of 1963 Steinberger and Rights published a work in which they demonstrated that following the intravenous immunization of rabbits the cells of the spleen become resistant to infection with

the virus. The authors consider that this resistance is specific, that is, it is manifested only in respect to the homologous virus and is not connected with antibodies.

In conclusion it is necessary to dwell on the problem concerning postinoculative complications.

According to the descriptive expression of Herrlich, "We have only one path in order to prevent smallpox -- to carry out inoculations, and only one path to avoid postinoculative complications -- to not carry out inoculations" (1958). In actuality complications, observed sometimes following vaccination against smallpox, remain one of the serious problems in the practice of smallpox vaccination. In the Soviet Union this problem still has not received the necessary attention. And besides this, statistics (unfortunately interrupted and incomplete) testify that postvaccination complications are observed here. Work on the prophylaxis of complications should be carried out extensively -- in experimental, clinical-therapeutic and organizational projects. Here it is necessary to have a thorough consideration and analysis of each case of complication. A difficulty is that the nature of the most serious complications, in particular postvaccination encephalites, remain unexplained up until the end. At the present time a number of methods have been proposed for the prevention of postvaccination encephalites; Inoculation of smallpox vaccine simultaneously with the intramuscular administration of human gamma-globulin with an increased content of antismallpox antibodies and the preliminary administration of inactivated smallpox vaccine. For treatment of postvaccination complications, specific gamma-globulin is used which is obtained from the serum of recently vaccinated persons or of animals which are hyperimmunized with the vaccine virus (Kempe, 1960; Marennikova, 1963). Apparently, a certain effect can be expected even from the new chemotherapeutic preparations of a thiosemicarbazone nature.

At the present time there is no doubt that even a timely vaccination does not always save a person from the disease. If the vaccination is made belatedly, then in the majority of cases it is noneffective. The investigations of recent years showed the feasibility of effective prophylactic intervention by the application, in addition to the vaccination, of human gamma-globulins of human (Kempe et al., 1961) and animal origin (Marennikova, 1963), and also of chemopreparations, in particular, N-methylisatin-beta-thiosemicarbazone.

The materials presented in the ~~present~~ article testify that in the problem of smallpox prophylaxis, in spite of the doubtless successes achieved, there remain many unresolved and unclear problems. Their rapid resolution will have great significance both for theoretical virology as well as for practical public health. ( )

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WORLD SMALLPOX INDICENCE

Continent	Index	Year 1959	1960	1961	1962	1963(11months)
Africa	Incidence	13,950	15,851	24,025	24,188	15,078
	Lethality	1,071	1,017	1,798	2,423	1,484
America	Incidence	4,889	3,090	1,939	3,029	241
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Asia	Incidence	58,085	39,221	53,549	46,374	72,973
	Lethality	15,781	9,639	13,081	12,287	24,033
Europe	Incidence	13	47	24	137	145
	Lethality	1	-----	4	27	11
All told	Incidence	76,937	58,209	79,537	73,728	88,437
	Lethality	16,853	10,656	14,883	14,737	25,544